Behavioural Brain Research 292 (2015) 266–277

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Gene and stress history interplay in emergence of PTSD-like features

Nabarun Chakraborty[a], James Meyerhoff[a], Aarti Gautam[a], Seid Muhie[a], Meskerem Jibitu[a], Thereza C.M. De Lima[b], Rasha Hammamieh[a,*], Marti Jett[a]

[a] US Army Center for Environmental Health Research, Fort Detrick, MD 21702-5010, USA
[b] Federal University of Santa Catarina – Department of Pharmacology, Florianopolis, SC, Brazil

HIGHLIGHTS

- Three mouse strains were subjected to a stress model eliciting PTSD-like features.
- Under stress, three strains demonstrated different phenotypic/genetic aspects.
- BALB/cj displayed delayed arousals of many phenotypic features.
- C57BL/6j showed signatures of poor cardiac health.
- Genetic heterogeneity and stress history contributed in PTSD prevalence.

ARTICLE INFO

Article history:
Received 12 January 2015
Received in revised form 9 April 2015
Accepted 22 May 2015
Available online 27 May 2015

Keywords:
Post-traumatic stress disorder (PTSD)
Mouse strain differences
Gene–environment model
Aggressor exposed model
Mouse behavior

ABSTRACT

Systematically distinguishing genetic liability from other contributing factors is critical for designing a preventive strategy for post-traumatic stress disorder (PTSD). To address this issue, we investigated a murine model exposing C57BL/6j, DBA/2j and BALB/cj mice to repeated stress via exposure to conspecific aggressors (Agg-E). Naïve mice from each strain were subjected to the proximity of aggressor (Agg) mice for 6 h using a 'cage-within-a-cage' paradigm, which was repeated for 5 or 10 days with intermittent and unpredictable direct contact with Agg mice. During the Agg-E stress, DBA/2j developed a different strategy to evade Agg mice, which potentially contributed to its phenotypic resilience to Agg-E stress. Although Agg mice inflicted C57BL/6j and BALB/cj with equivalent numbers of strikes, BALB/cj displayed a distinct behavioral phenotype with delayed exhibition of a number of PTSD-like features. By contrast, C57BL/6j mice displayed unique vulnerability to Agg-E stress induced myocardopathy, possibly attributable to their particular susceptibility to hypoxia. A group of genes (*Bdnf*, *Ngf*, *Zwint*, *Cckbr*, *Slc6a4*, *Fkbp5*) linked to PTSD and synaptic plasticity were significantly altered in C57BL/6j and BALB/cj Agg-E mice. Contributions of Agg-E stress history and genotypic heterogeneity emerged as the key mediators of PTSD-like features. Linking genetic components to specific phenotypic and pathological features could have potential clinical implications.

1. Introduction

Trauma exposure is a necessary, but not sufficient condition for developing PTSD, which may explain why only a sub-population of trauma survivors develops PTSD. The prevalence of PTSD appears to be mediated by genetic predisposition putatively vulnerable to the environmental factors [1,2]. The gene–environment interaction model explains the higher PTSD prevalence among monozygotic twins as compared to their dizygotic counterparts exposed to equivalent traumatic burdens [3–5]. Similarly, higher PTSD prevalence was reported among the offspring of holocaust survivors [6] and of families with troubled histories [7]. In the context of accumulating evidence suggesting that PTSD could be a high-to-moderately heritable disease, molecular markers independent of environmental effects could have significant clinical importance [8–10]. However, finding appropriate cohort of controls [1,8] and controlling for magnitude of traumatic burden remain major challenges, as environmental interference can potentially overwhelm genetic liability [11].

Addressing these challenges requires in vivo investigations [12]. Inter-strain genetic heterogeneity and the histological and endocrinological variables are often considered to be the mediator of the strain-to-strain endophenotypic variances in mice. The inter-strain variability reflected in the sizes of the hippocampus

* Corresponding author at: US Army Center for Environmental Health Research, 568 Doughten Drive, Fort Detrick, MD 21702-5010, USA. Tel.: +1 3016197629; fax: +1 3016197606.
E-mail address: Rasha.Hammamieh1.civ@mail.mil (R. Hammamieh).

http://dx.doi.org/10.1016/j.bbr.2015.05.038
0166-4328/

Report Documentation Page

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
14 SEP 2015	**research**	**01-01-2012 to 01-12-2014**

4. TITLE AND SUBTITLE
Gene and stress history interplay in emegence of PTSD-like features

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Nabrun Charaborty; James Meyeroff; A Gautam; Seid Muhie; Meskerem Jibitu

5d. PROJECT NUMBER

5e. TASK NUMBER
4.0

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
U.S.Army Center for Environmental Health Research,568 Doughten Drive,Fort Detrick,MD,21702-5010

8. PERFORMING ORGANIZATION REPORT NUMBER
14-36

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command, 504 Scott Street, Fort Detrick, MD, 21702-5010

10. SPONSOR/MONITOR'S ACRONYM(S)
USAMRMC

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES
3 mouse strains were subjected to stress model.

14. ABSTRACT

Systematically distinguishing genetic liability from other contributing factors is critical for designing a preventive strategy for post-traumatic stress disorder (PTSD). To address this issue, we investigated a murine model exposing C57BL/6j, DBA/2j and BALB/cj mice to repeated stress via exposure to conspeci???c aggressors (Agg-E). Na??ve mice from each strain were subjected to the proximity of aggressor (Agg) mice for 6 h using a ???cage-within-a-cage??? paradigm, which was repeated for 5 or 10 days with intermittent and unpredictable direct contact with Agg mice. During the Agg-E stress, DBA/2j developed a different strategy to evade Agg mice, which potentially contributed to its phenotypic resilience to Agg-E stress. Although Agg mice in???icted C57BL/6j and BALB/cj with equivalent numbers of strikes, BALB/cj displayed a distinct behavioral phenotype with delayed exhibition of a number of PTSD-like features. By contrast, C57BL/6j mice displayed unique vulnerability to Agg-E stress induced myocardopathy, possibly attributable to their particular susceptibility to hypoxia. A group of genes (Bdnf, Ngf, Zwint, Cckbr, Slc6a4, Fkbp5) linked to PTSD and synaptic plasticity were signi???cantly altered in C57BL/6j and BALB/cj Agg-E mice. Contributions of Agg-E stress history and genotypic heterogeneity emerged as the key mediators of PTSD-like features. Linking genetic components to speci???c phenotypic and pathological features could have potential clinical implications.

15. SUBJECT TERMS
Post-traumatic stress disorder (PTSD); Mouse strain differences; Gene???environment model; Aggressor exposed model; Mouse behavior

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT **unclassified**	b. ABSTRACT **unclassified**	c. THIS PAGE **unclassified**	**Same as Report (SAR)**	**12**	

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std Z39-18

[13], nucleus accumbens [14] and neocortical elements [15], as well as the diversities in the cholinergic [16] and dopaminergic [17] feedbacks could contribute to social and cognitive plasticity [18,19].

Hence, the present study is focused on characterizing the contributions of genetic heterogeneity and environmental challenges in developing PTSD-like features elicited by an agonistic social situation including both threat and attack. As developed by our group [20], the Agg-E repeated stress model attempts to emulate the combat-like situation, where soldiers are exposed to grave threats repeatedly and uncontrollably. Arguably combat-related PTSD symptoms could differ from those of civilian PTSD patients. Our model concurred with the etiological and face validators mandated for a PTSD rodent model [12]. Using C57BL/6j mice as Agg-E cohort, this model characterized the genomics [21,22] and metabolomics [23] signatures of PTSD. The present model utilizes SJL male mice trained to be aggressive toward the intruders; hence unlike the typical social stress models [24,25], the present model limited the direct intruder–resident interactions to not more than one minute; longer direct interaction could cause serious and undesired injury in Agg-E mice. Such aggressive interaction is designed to make the Agg-E mice encounters as repeated life-threatening events, a critical feature of a combat theater. Typical PTSD models, such as the brief footshock model [14,26] and the predator stress model [27] fails to emulate certain key features of combat theater such as the repeated, random and uncontrolled occurrence of the potentially life-threatening events and the conspecific interactions, respectively.

Nevertheless, all of these models demonstrated the interplay of gene and environment in developing fear responses and other key PTSD-like features. Both the magnitude and characteristics of the traumatic load play a major role in determining the strain-to-strain differences in the stress adaptation strategies. For instance, the social stress model elicited acute social avoidance in BALB/cj in comparison to C57BL/6j [25]. The single foot shock augmented social impairment in DBA/2j and behavioral "depression" in C57BL/6j [28] accompanied by diminished contextual fear conditioning [14]. The predator stress model [29] found BALB/c mice more resilient than C57BL/6 and DBA/2 strains. With exposure to the contextual threat, all three strains behaved equivalently; but shifting the cues' character from the feline feces to clay triggered anxiogenic-like responses in C57BL/6 and DBA/2 mice more than in the BALB/c strain. Conversely, a set of heterogeneous stressors composed of open field, elevated maze and exposure to novel objects elicited higher fear responses in BALB/cj compared to C57BL/6j [30]; and DBA/2j performed better than C57BL/6j in accomplishing passive avoidance tasks, but not in complex tasks [31].

Motivated from these studies, we focused on characterizing the implications of the genetic heterogeneity among three strains of mice (C57BL/6j, DBA/2j, and BALB/cj) responding to the Agg-E stress, eliciting PTSD-like behaviors. The phenotypic variability along with the expressions of a selective genomic panel associated with PTSD and synaptic plasticity were evaluated.

2. Material and methods

2.1. Mice obtained for the study

We purchased four strains of male mice, SJL, C57BL/6j, DBA/2j and BALB/cj, from Jackson Laboratory (Bar Harbor, ME), all at the age of six weeks and weighing 20–22 g. Mice were kept in a temperature-controlled room ($21 \pm 2\,^{\circ}C$) on reverse 12 h/12 h light/dark cycle (lights off at 6:00 AM) and all experiments were conducted during the dark phase of dark/light cycle.

SJL albino mice (Agg mice) were trained to reliably and quickly initiate aggressive behavior against the intruder Agg-exposed mice (Agg-E mice) following a protocol described by our previous communication [20]. A separate room was dedicated exclusively for the other three strains: C57BL/6j, DBA/2j and BALB/cj. Mice were single-housed in polycarbonate cages (48 cm × 27 cm × 20 cm) for at least one month before initiating the protocol.

All animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC) at the Walter Reed Army Institute of Research, Silver Spring, MD, and the Medstar Research Institute, Washington Hospital Center, Washington, DC, and were performed in a facility accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care International (AAALAC).

2.2. Agg-E stress sessions

We described the detailed protocol in an earlier communication [20]. Briefly, the Agg-E stress session was conducted during the dark light cycle. We caged the Agg-E mouse inside a small wire mesh cage (17.5 cm × 14 cm × 7.5 cm), which was placed inside the Agg's polycarbonate home cage for 6 h (9:00 AM–3:00 PM) without any access to food or liquid (6 h 'cage-within-cage' confinement). The Agg mouse had nourishment ad libitum and free access around the mesh cage. In addition to the indirect exposure, we also directly exposed the 'intruder' Agg-E mice to the 'resident' Agg mice two to three randomly selected times per day (d). Each bout of direct exposure lasted for 1 min or until 10 strikes were inflicted on the Agg-E mouse, whichever came first. In a separate room, the control mice were kept in the same type of 6 h 'cage-within-cage' confinement with fresh bedding for 6 h without any proximity or exposure to Agg mice. After the 6 h confinement, the controls and Agg-E mice were returned to their respective single-housing home cages with food and liquid ad libitum until the start of next day's 6 h sessions, which were repeated daily for either 5 or 10 consecutive days, creating 5-day and 10-day stress groups, respectively.

We randomized the controls and Agg-E mice subjected to 5-day Agg-E sessions into two groups; one of the two groups was sacrificed 1 day after the 5-day Agg-E session and the other group's sacrifice was delayed by 1.5 weeks (w). Similarly, the controls and Agg-E mice subjected to 10-day Agg-E sessions were sacrificed either 1 d or 4 w post Agg-E sessions. Mice spent their respective 1.5 w or 4 w delay intervals singly housed inside their home cages with food and water ad libitum.

2.3. Measurement of bodyweight, temperature and urine markings

The detailed protocol for recording this set of physiological information was published in an earlier report [20]. Briefly, *bodyweight* was recorded before (Fig. S2A) and after (Fig. 1A) each 6 h 'cage-in-cage' confinement as were the body *temperatures* (Fig. S2B–C). The core body temperatures were recorded using Electronic ID TranspondersTM (IPTT-300, Bio Medic Data Systems Inc., DE) subcutaneously implanted into the dorsal cervical region of the mice 3 d in advance of the first stress session. Territorial distributions of *urine marking* were recorded using blotting papers (0.8 mm thickness) placed daily under the mesh cages of controls and Agg-E mice during 6 h 'cage-in-cage' confinement (Fig. 1B). The papers were UV-scanned using Molecular Imager Fx® (BioRad, Hercules, CA) and the number of urination marks and the areas contouring the urine markings were measured using Quantity One® software (BioRad, Hercules, CA).

2.4. Behavioral assessment during the direct exposures of Agg-E to Agg mice

We visually inspected the direct interactions between the Agg and Agg-E mice. Trained technicians counted and timed the bouts

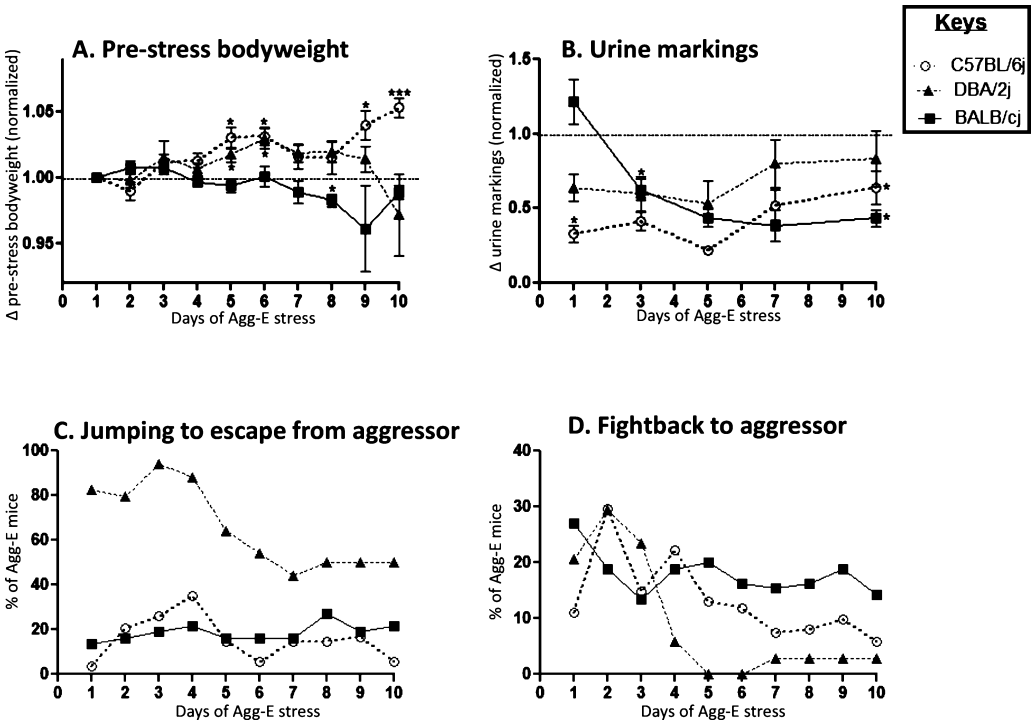

Fig. 1. Phenotypes of Agg-E mice during the Agg-E sessions. All records were taken during the 5-day/10-day Agg-ExS ($N = 16–21$ mice/group); Welch's t-test: $^{***}p < 0.001$, $^{*}p < 0.05$.
In A and B, the horizontal dotted line passing through 1.0 of Y-axis marks the baseline defined by the normalized phenotypes exhibited by the control mice. Mean ± SEM of Agg-E mice divided by the strain-specific controls (Agg-E mice/control) are plotted against each day point. (A) Pre-stress bodyweight. (B) Urine markings.
In C and D, behaviors were monitored during the daily bouts of direct exposures; the fractions of the total Agg-E mice group are plotted against each day point. (C) Jumping to escape from aggressor. (D) Fightback to aggressor.

of strikes, and monitored three behavioral phenotypes of the Agg-E mice described in Table 1, namely *jumping* (Fig. 1C), *freezing* (Fig. S2D) and *fightback* (Fig. 1D).

2.5. Cardiac histological analysis

Following a protocol previously described [20], heart tissues collected from the sacrificed mice were perfused in ice-cold 4% paraformaldehyde. Tissues were then stained, sliced, and mounted, and a board-certified veterinary pathologist, unaware of the animal's behavioral history or treatment group, analyzed the samples using bright field optics (Table 2).

2.6. Phenotypic evaluation during the partition test

The partition test [20] was designed to present the contextual cues reminiscent of the past agonistic interactions between the Agg and Agg-E mice. We performed this test 1 d and 1.5 w after the 5-day Agg-E session and 1 d and 4 w after the 10-day Agg-E session. We divided the soiled Agg home cage with a plastic fenestrated partition and placed one Agg and one Agg-E mice on opposite sides of the partition for 5 min. The partition permitted the passage of sensory cues but prevented direct physical contact. The ethogram of the Agg-E and control mice was recorded and the videos were analyzed by Ethovision XT v.7 software (Noldus®, Leesburg, VA) at a rate of 15 samples per second using dynamic subtraction detection, with the object always darker than background, erosion and dilation filters of one pixel, and one sample interval for averaging filter [20].

Table 1 describes the behavioral parameters we evaluated herein. For measurement purposes, we hypothetically divided the side of the cage temporarily holding the Agg-E mouse (or the control) into three zones (Fig. S1). The six inch wide zone adjacent to the partition was defined as the "partition zone" and the six inch wide region across the three side walls at the rear was defined as the "peripheral zone". The central part of the cage surrounded by the partition and peripheral zones was defined the "mid zone". We defined the frequency of time spent in the partition zone as the "*partition vigilance*" and the ratio of the time spent between the peripheral and partition zone as the "*withdrawal toward peripheral region*". "*Grooming*" monitored visually by an observer is a complex sequence of licking of paws and washing of nose, head and body. "*Retarded locomotion*" refers to the fraction of total movement that happened inside the partition zone and "*freezing*" refers to an event of immobility displayed inside the partition zone when all visible movement of the body except the movements for respiration became non-existent. "*Overall activity*" is defined as the average velocity of the mice recorded during the 5 min test in all three zones. Our previous communication [20] discussed all of these phenotypic endpoints in detail. The behavioral parameters of the Agg-E mice (Fig. 2 and Fig. S3) were normalized by their age- and strain-matched controls as described earlier [20]. Present strategy potentially minimized the behavioral variances attributed to the non-Agg-E stress-mediated factors, such as age, food/liquid restriction, movement restriction and prolonged social isolation in order to extract the unique contributions of Agg-E repeated stress.

2.7. Hemibrain isolation and downstream qPCR analysis

Animals were euthanized by cervical dislocation 1 h after the partition test. Left hemibrains were collected from the mice after euthanization and were immediately snap-frozen. The samples were archived in a −80 °C freezer for long term storage until the day of nucleic acid isolation using Trizol (Life Technologies, Carlsbad, CA) following the vendor's recommendations.

Table 1
Descriptions of the behavioral phenotypes monitored and measured during the direct exposure to Agg (Agg-E) and during the 5-min partition test.

	Phenotype	Description
During direct exposure	*Jumping* to escape from Agg	The sudden and vigorous leap performed by the Agg-E mouse as an apparent attempt to evade the Agg-initiated attack.
	Fightback to Agg	A defensive aggression trait displayed by the Agg-E mouse. Triggered by the Agg-initiated attack, the Agg-E mouse first rose up on its hind limbs in a "boxing" posture and swiftly struck out using a forepaw toward the approaching Agg. This appears to be a defensive-aggression mechanism closely resembling the fightback phenotype demonstrated by the male rats stressed by shock [75].
	Freezing	The period of immobility shown by Agg-E mouse as the absence of all visible movement of the body except the movement for respiration
During partition test	*Partition vigilance*	The total time spent per visit to the partition zone. The six inch wide area in front of the subject animal's side of partition is marked as the "partition zone". This parameter may also imply the inverse of the partition avoidance. The earful mouse is expected to show suppressed vigilance.
	Withdrawal to peripheral region	The time spent at the cage peripheral zone in comparison to the time spent at the partition zone (duration of time spent in peripheral zone/partition zone). The peripheral region is marked by the six inch wide area across the three sidewalls of the cage used for residing the subject mouse. The fearful mouse is expected to spend more time in periphery.
	Reduced locomotion	The ratio of the distance traveled inside the partition zone vs distance traveled the total cage portion available to the subject mouse during partition test. Reduced locomotion is expected to be pronounced in stressed animals.
	Freezing	The period of immobility defined by <10% shift of body position between sequential frames of the fifteen frames sampled per second. The ratio of freezing duration and total time in partition zone is presented. Freezing is believed to be positively correlated with fear response.
	Grooming	A behavioral endpoint consists of licking of paws and followed by stroking of nose, head and body that may occur in any order. Total grooming duration presented herein is measured by visual inspection of the videos. It is believed to be a common response to fear.
	Overall activity	The average velocity of the mice during the partition test.

Table 2
The fraction (in percentage) of the total Agg-E population showing myocardial degeneration and lymphohistiocytic myocarditis.

Strain		5-day Agg-E stress (%)		10-day Agg-E stress (%)	
		1 d delay	1.5 w delay	1 d delay	4 w delay
C57BL/6j	Myocardial Degeneration	12.5	60.0	36.4	20.0
	Lymphohistiocytic Myocarditis	75.0	None	45.5	None
DBA/2j	Myocardial Degeneration	None	None	None	None
	Lymphohistiocytic Myocarditis	None	None	None	None
BALB/cj	Myocardial Degeneration	36.4	None	None	None
	Lymphohistiocytic Myocarditis	None	None	None	None

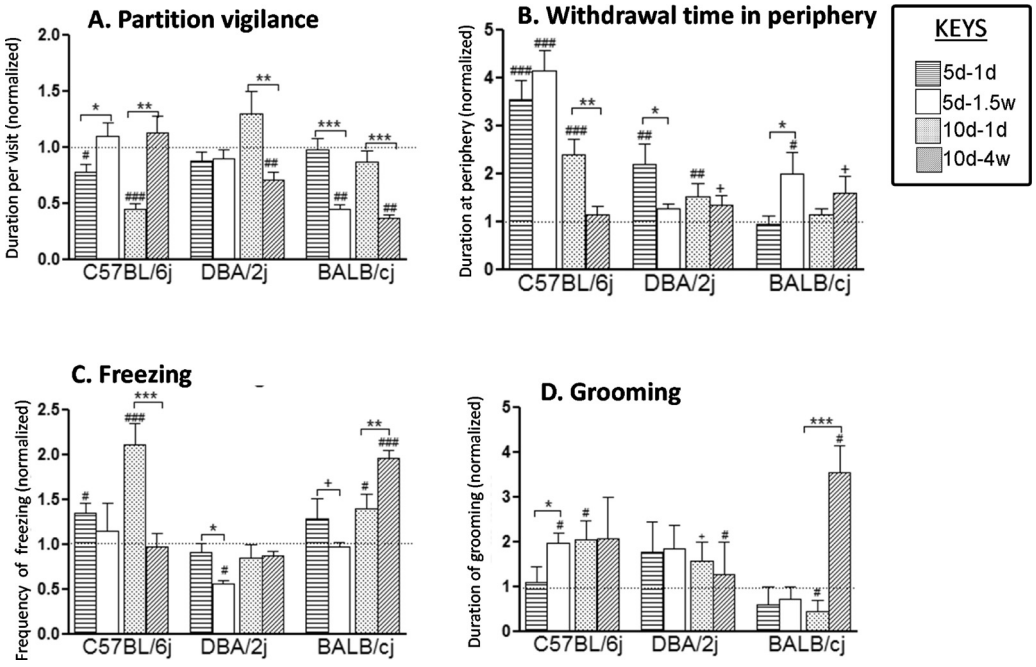

Fig. 2. Ethogram of Agg-E mice during the partition test. The endpoints of Agg-E mice recorded during the partition test are normalized against the age- and strain-matched controls (Agg-E mice/control) and the mean ± SEM of Agg-E mice are plotted. The horizontal dotted line passing through 1.0 of *Y*-axis marks the baseline defined by the normalized phenotypes exhibited by the control mice. Welch's *t*-test compared Agg-E mice vs. Control: $^{###}p < 0.001$, $^{##}p < 0.01$, $^{#}p < 0.05$, $^{+}p < 0.1$; and Welch's *t*-test compared two delay periods (1 d vs. 1.5 w for 5-day Agg-E stress and 1 d vs. 4 w 10-day Agg-E stress): $^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$, $^{+}p < 0.1$ ($N = 6$–17 mice/group). (A) Partition vigilance, (B) Withdrawal time in periphery, (C) Freezing, (D) Grooming.

Real-time PCR was carried out using the RT^2 Profiler PCR Array System (SA Biosciences, Frederick, MD) on the ABI 7900HT platform (Life Technologies) following the protocol described earlier [32].

2.8. Statistical analysis

Statistical analyses were performed using GraphPad® version 5.0 software (GraphPad Software, Inc., La Jolla, CA). Experimental results were represented as mean ± SEM. As per the earlier description [20,33], fitting models were used to evaluate the longitudinal trajectories of the phenotypes (such as body weight, temperature, and urine markings) observed over the 5-/10-day Agg-E sessions. The robustness of fit of the curve was measured by r^2 and the p value estimated the degree of deviation of the fitting curve's slope. We limited the reporting to the linear regression model that essentially explained most of the longitudinal trajectories.

Unpaired t-tests with Welch's correction were used to evaluate the shift of the physiological and phenotypic endpoints. Pair-wise associations between phenotypic parameters were evaluated using the Spearman correlation. Two-way and 3-way analysis of variance (ANOVA) and multivariate analysis of variance (MANOVA) were performed. The principal component analysis (PCA) [34–39] is reported in the supplementary data.

We used *R* package version 3.0.0 (http://cran.r-project.org) to compute PCA and other statistical analysis, and plotted the variables using Biplot. The loading factors contributing most significantly to the top three principal components were computed by ANOVA and MANOVA. Similar schemes were used in the past to explain the ethogram [34–39].

We used $p < 0.05$ as the significance threshold for all the calculations.

3. Results

3.1. Strain-to-strain variances in the longitudinal shift of the bodyweight, temperature *and* urine markings *during the Agg-E stress session*

We measured the *bodyweights* (Fig. 1A and Fig. S2A) and *temperatures* of the Agg-E mice (Fig. S2B–C) before (pre-stress) and after (post-stress) the 6 h 'cage-within-cage' confinement, and the *urine markings* (Fig. 1B) were measured during the 6 h 'cage-within-cage' confinement. The pre-stress bodyweight and temperature defined the baselines of the respective studies. The deviations of *bodyweight* and *temperature* from their respective baselines were normalized by the controls (Agg-E/control mice) and the daily averages of the normalized data (±SEM) were plotted (N = 5–21 mice per group). Pair-wise Welch's t-test computed the differences between control and Agg-E mice at individual time points.

The linear regression model was used to interpret the longitudinal trend of changing the phenotypes as per earlier reports [20,33,40]. C57BL/6j showed a gradual and significant weight gain in the course of a 10-day Agg-E session; in contrast, BALB/cj displayed a relatively descending trend in weight significantly different from C57BL/6j (Fig. 1A).

A strain-to-strain difference was also evident in the longitudinal shift of the post-stress *bodyweights* of Agg-E mice (Fig. S2A). C57BL/6j Agg-E mice showed a sharp weight gain on day 5. Showing an opposite trend, the *bodyweights* of BALB/cj Agg-E mice displayed an early loss on day 4 followed by a regain up to the baseline. DBA/2j mice showed a significant linear inclination of post-stress *bodyweight* over the 10 days.

Unlike the *bodyweight* profile, the body *temperature* was found to be unrelated to the Agg-E stress (Fig. S2B–C) although other studies have reported the alteration of core body temperature as a consequence of emotional disturbances [41,42]. Nevertheless, a significant strain-to-strain difference was registered in the longitudinal alteration of pre-stress *temperature* of Agg-E mice.

Examining the *urine markings* on the blotter papers, we observed strain-to-strain differences (Fig. 1B) in territorial behavior in course of the 10-day Agg-E sessions. We normalized the daily urine counts of the Agg-E mouse by its strain-specific controls (Agg-E/Control). The numbers of *urine markings* of the Agg-E mice of C57BL/6j and DBA/2j increased with time. BALB/cj Agg-E mice, on the other hand, showed a steady decline of the territorial behavior with a terminal plateau. At day 10, the territorial behavior of C57BL/6j and BALB/cj were significantly different from their respective controls as computed by Welch's t-test.

3.2. Strain-to-strain variability in adopting strategies to evade Agg mice

Fig. 1C–D and Fig. S2D depict three ethograms manually recorded during the daily bouts of direct exposures in the course of 10-day Agg-E sessions. An individual Agg-E mouse displaying a particular trait in at least two of three direct exposures per day was classified positive for that day. The longitudinal trend of the behavioral shift was robustly explained by the linear regression model.

3.2.1. Jumping (Fig. 1C)

A significant strain-to-strain variability was evident in adopting jumping as a mode to escape from the Agg mice. In comparison to C57BL/6j and BALB/cj, 30%–60% more DBA/2j Agg-E mice displayed jumping, respectively. During the final two days of the 10-day Agg-E session, 40% of DBA/2j Agg-E mice displayed a rate of jumping, which was twice that of the other two strains.

In fact, DBA/2j Agg-E mice were possibly able to avoid the attacks from the Agg mice due to their ability to jump so frequently. On average, Agg mice were able to inflict only 6 strikes/min on DBA/2j Agg-E mice; whereas they inflicted 10 strikes on C57BL/6j and BALB/cj Agg-E mice within 48 s during the direct exposures. The gradual elevation of the post-stress body weights of DBA/2j Agg-E mice (Fig. S2A) was oppositely and significantly correlated with the diminishing tendencies of jumping ($r = -0.65$).

3.2.2. Fightback (Fig. 1D)

Fightback to the Agg mouse was another phenotypic endpoint commonly displayed by all three strains, though a significant variability was noted among the three strains. This trait was gradually and significantly diminished in C57BL/6j and DBA/2j; while the size of BALB/cj subpopulation displaying *fightback* remained same. The longitudinal elevation of the pre-stress *bodyweights* of C57BL/6j Agg-E mice was negatively correlated with its declining *fightback* tendency ($r = -0.65$). The *fightback* tendencies' contrasting correlations with the territorial behaviors of DBA/2j and BALB/cj were noteworthy. In DBA/2j, the declining *fightback* tendency was negatively correlated ($r = -0.94$) with its increasing territorial behavior, whereas in BALB/cj *fightback* tendency and territorial behavior showed positive correlation as both decreased over time ($r = 0.86$).

3.2.3. Freezing (Fig. S2D)

We observed significant strain-to-strain differences in *freezing* behavior. In fact, the size of population displaying *freezing* significantly decreased with time across all three strains of mice. The declining tendency of *freezing* was positively correlated with the decreasing post-stress *bodyweights* of BALB/cj Agg-E mice ($r = 0.72$), but negatively correlated with the increasing post-stress *bodyweights* of DBA/2j Agg-E mice ($r = -0.68$).

In the case of DBA/2j Agg-E mice, their *jumping*, *fightback* and *freezing* behaviors decreased over time in significantly

correlated fashions: r (*jumping* vs. *fightback*) = 0.74; r (*jumping* vs. *freezing*) = 0.90; r (*fightback* vs. *freezing*) = 0.58. The jumping and *fightback* tendencies of C57BL/6j Agg-E mice diminished in a correlated fashion (r = 0.60). Interestingly, no behavioral traits showed significant correlation in BALB/cj Agg-E mice.

3.3. Assessment of the contributing factors defining the behavioral plasticity during the partition test

We hypothesized that the post-stress behavioral plasticity was primarily modulated by the three following factors: (i) the strain differences or the genotypic heterogeneity defined by Strain (C57BL/6j, DBA/2j and BALB/cj), (ii) the magnitude of Agg-E stress defined by Stress (controls vs. Agg-E mice), and (iii) the duration of the delay period defined by Delay (1 d and 1.5 w post 5-day Agg-E session, and 1 d and 4 w post 10-day Agg-E session). Theoretically, the potential roles of undetermined additional factors in mediating the behavioral plasticity cannot be dismissed; however their impacts should be insignificant due to the carefully controlled design of this study. Together, the Stress and Delay were defined as the Stress history.

Two-way ANOVA (Table 3 and Table S1A) captured the independent and interactive impacts of strain differences and the Stress history. Deconstructing the Stress history into two independent univariates, three-way ANOVA (Table 3 and Table S1B) captured the independent and interactive contributions of all three variables of interest. MANOVA explained their interactive contribution by manipulating the three as dependent variables (Table 3 and Table S1C).

We also computed the impact of Stress and Delay on all three individual mouse strains, since the interplay of these two variables defines the onset of PTSD-like features [20,43]. We previously reported the corresponding result manifested only by C57BL/6j male mice [20].

Pair-wise Welch's t-test computed the differences between control and Agg-E mice measured at different time points. Corresponding PCA analysis is reported in the supplementary data.

3.3.1. Partition vigilance (Fig. 2A, Table 3)

5-day Agg-E session vigilance: One day after the 5-day Agg-E session, Agg-E mice of C57BL/6j showed significantly reduced *partition vigilance*, but 1.5 w delay significantly increased the duration per visit to the partition. Agg-E mice of DBA/2j and BALB/cj strains showed control-like vigilance 1 d after 5-day Agg-E sessions. Subsequent 1.5 w delay did not alter the vigilance tendencies of DBA/2j Agg-E mice, but significantly declined among the BALB/cj Agg-E mice.

The variability in *partition vigilance* was explained by the interplay of mouse Strain and the Stress history. The exclusive impact of Stress, the interactive impact of Strain and Delay, and the comprehensive interaction among all three factors also emerged as significant.

MANOVA found no significant interactive impact of the three variables acting dependently.

Considering the three strains individually, the interplay between Stress and Delay emerged as nearly significant in C57BL/6j [20] and BALB/cj Agg-E mice, but not in DBA/2j Agg-E mice.

10-day Agg-E session vigilance: 1 d after the 10-day Agg-E session, the *partition vigilance* was significantly suppressed among the Agg-E mice of C57BL/6j as compared to controls. The 4 w delay significantly increased the vigilance, returning it to the control level. The Agg-E mice of DBA/2j and BALB/cj displayed control-like *partition vigilance* 1 d after the 10-day Agg-E sessions, which declined significantly with Delay.

The impacts of Strain, Stress history, and their interplay were significant. Three-way ANOVA further explained significant contributions from Stress, Strain × Stress and Strain × Delay, and moderate contributions from Delay and its interplay with Stress (Stress × Delay).

MANOVA analysis suggested significant interactive influence of all three dependent variables.

Considering the three strains individually, the interplay between Stress and Delay emerged as highly significant in the Agg-E mice of C57BL/6j [20] and moderately significant in BALB/cj and DBA/2j.

3.3.2. Withdrawal time in periphery (Fig. 2B, Table 3)

5-day Agg-E session withdrawal time: C57BL/6j Agg-E mice spent significantly longer *withdrawal time in periphery* 1 d and 1.5 w after the 5-day Agg-E session.

The withdrawal tendency to the peripheral region of DBA/2j Agg-E mice was twice that of the controls 1 d after the 5-day Agg-E session and was significantly reduced with the subsequent 1.5 w delay. In sharp contrast, BALB/cj Agg-E mice showed only minimal withdrawal traits 1d after the 5-day Agg-E session, but the withdrawal time was increased significantly after 1.5 w delay.

The variance of this behavioral parameter was explained by the exclusive and interactive effects of Strain and Stress history. Three-way ANOVA found significant impact from the combined effect of three interactive factors along with the exclusive impact of Stress, and its interplay with Delay.

MANOVA analysis suggested significant interactive influence of all three dependent variables.

Considering the three strains individually, the interplay between Stress and Delay emerged as highly significant in Agg-E mice of C57BL/6j [20], moderately significant in BALB/cj, but non-significant in DBA/2j.

10-day Agg-E session withdrawal time: One day after the 10-day Agg-E session, C57BL/6j Agg-E mice spent significantly longer *withdrawal time in periphery*; the trait, however, was reduced by 50% after the 4 w delay. Agg-E mice of the DBA/2j strain spent consistently more time in the periphery than the controls 1 d and 4 w after the 10-day Agg-E session. A late marginally significant increment of withdrawal time to the periphery was recorded for BALB/cj Agg-E mice.

The exclusive impact of the Stress history and its interplay with Strain emerged significant. The contribution from Stress history was primarily explained by Stress. The interplay between Strain and Delay and the combined interplay among all three variables was significant.

MANOVA analysis suggested that there was significant interactive influence of all three dependent variables.

Considering the three strains individually, the interplay between Stress and Delay emerged as highly significant in Agg-E mice of C57BL/6j [20], but non-significant in BALB/cj and DBA/2j.

3.3.3. Freezing (Fig. 2C, Table 3)

5-day Agg-E session freezing: The Agg-E mice of C57BL/6j froze more frequently than the control 1 d after the 5-day Agg-E session, but displayed only control-like *freezing* 1.5 w post 5-day Agg-E session. The Agg-E mice of DBA/2j and BALB/cj displayed control-like *freezing* 1 d after 5-day Agg-E session. Delay, however, caused a significant reduction of *freezing*.

Only the exclusive contribution of Strain emerged as significant. MANOVA found non-significant contribution.

Considering the three strains individually, the interplay between Stress and Delay emerged as non-significant across the strains.

10-day Agg-E session freezing: The Agg-E mice of C57BL/6j showed two-fold higher *freezing* than the controls 1 d after the 10-day Agg-E session; and 4 w delay diminished their *freezing* durations to control levels. The *freezing* durations of Agg-E mice of

Table 3
ANOVA analyses of the behavioral parameters investigated during the partition test.

Factor	Partition vigilance		Withdrawal time in periphery		Freezing		Grooming		Reduced locomotion		Overall activity	
	p value	*F* value	*p* value	*F* value	*p* value	*F* value	*p* value	*F* value	*p* value	*F* value	*p* value	*F* value
2-way ANOVA												
5-day Agg-E stress followed by 1 d or 1.5 w delay												
Strain	–	–	<0.0001	25.03	0.04	3.23	–	–	–	–	–	–
Stress history	–	–	<0.0001	16.91	–	–	–	–	0.001	3.93	–	–
Strain × Stress history	0.02	2.57	<0.0001	7.09	–	–	–	–	–	–	*0.07*	*1.96*
10-day Agg-E stress followed by 1 d or 4 w delay												
Strain	0.02	3.88	–	–	<0.0001	11.70	–	–	–	–	–	–
Stress history	0.002	5.07	0.0005	6.37	0.0001	7.17	0.0008	5.86	0.0001	7.27	–	–
Strain × Stress history	0.0003	4.53	0.03	2.42	<0.0001	7.39	0.01	2.88	–	–	–	–
3-way ANOVA												
5-day Agg-E stress followed by 1 d or 1.5 w delay												
Strain	–	–	<0.0001	25.03	0.04	3.23	–	–	–	–	–	–
Stress	0.04	4.28	<0.0001	22.16	–	–	–	–	0.001	11.27	0.03	5.04
Delay	–	–	–	–	–	–	–	–	–	–	–	–
Strain × Stress	–	–	–	–	0.05	2.97	–	–	–	–	*0.09*	*2.47*
Strain × Delay	0.05	2.99	0.04	3.42	–	–	–	–	–	–	–	–
Stress × Delay	–	–	–	–	–	–	–	–	–	–	–	–
Strain × Stress × Delay	0.03	3.48	0.03	3.46	–	–	–	–	–	–	–	–
10-day Agg-E stress followed by 1 d or 4 w delay												
Strain	0.01	11.71	–	–	<0.0001	11.70	–	–	–	–	–	–
Stress	0.004	8.53	<0.0001	20.17	<0.0001	21.47	0.0007	11.86	<0.0001	21.79	–	–
Delay	*0.08*	*3.17*	–	–	–	–	–	–	–	–	–	–
Strain × Delay	0.01	4.52	–	–	<0.0001	10.29	–	–	–	–	–	–
Strain × Delay	0.005	5.33	0.01	4.38	0.003	6.22	0.0004	8.34	–	–	–	–
Stress × Delay	*0.09*	*2.90*	–	–	–	–	0.02	5.77	–	–	–	–
Strain × Stress × Delay	0.006	5.18	0.02	4.24	0.004	5.74	*0.07*	*2.65*	–	–	–	–

DBA/2j did not change from controls 1 d and 4 w after 10-day Agg-E session. BALB/cj Agg-E mice displayed a modestly long *freezing* duration 1 d after 10-day Agg-E session, which was increased significantly after the 4 w delay.

The exclusive and interactive impacts of Strain and Stress history emerged as significant. The interplay of Strain with Stress and Delay respectively emerged as significant; in addition, the combinatory influence of the three variables was significant.

MANOVA analysis suggested significant interactive influence of all three dependent variables.

Considering the three strains individually, the interplay between Stress and Delay emerged as highly significant in Agg-E mice of C57BL/6j [20] and BALB/cj; but non-significant in DBA/2j.

3.3.4. Grooming (Fig. 1D, Table 3)

5-day Agg-E session grooming: Among three mouse strains, only the Agg-E mice of C57BL/6j showed a delay-induced significant shift in *grooming* duration.

None of variables was significant in interpreting *grooming* as inferred from 2-/3-way ANOVA. MANOVA analysis, however, suggested modest interactive influence of all three dependent variables.

Considering the three strains individually, the interplay between Stress and Delay emerged as non-significant in modulating *grooming* across the strains.

10-day Agg-E session grooming: Grooming times of Agg-E mice of both C57BL/6j and DBA/2j strains were nearly significantly higher than the controls 1 d and 4 w post 10-day Agg-E session. On the other hand, the Agg-E mice of BALB/cj strain spend significantly less time *grooming* than the controls 1 d after the stress. After 4 w delay, however, the grooming time significantly increased in BALB/cj.

The exclusive impact of Stress history and its interplay with Strain emerged as significant. The contribution from Stress history was primarily explained by Stress. The interplay between Strain and Delay and the combinatory influence of all three factors were significant.

MANOVA analysis suggested significant interactive influence of all three dependent variables.

Considering the three strains individually, the interplay between Stress and Delay emerged as highly significant in BALB/cj and non-significant in C57BL/6j and DBA/2j.

3.3.5. Reduced locomotion (Fig. S3A, Table 3)

5-day Agg-E session locomotion: The Agg-E of C57BL/6j showed significantly decreased *locomotion* 1 d after 5-day Agg-E session. The Agg-E mice of DBA/2j displayed control-like locomotion both 1 d and 1.5 w after the 5-day Agg-E session. The Agg-E mice of the BALB/cj strain, on the other hand, while normal at 1 d, displayed significantly less locomotion after 1.5 w delay. Thus, the stress-induced inhibition of locomotion was delayed in BALB/cj, compared to the other two strains.

MANOVA analysis found significant interactive contribution among all three dependent variables.

Considering the three strains individually, the interplay between Stress and Delay emerged as highly significant in BALB/cj Agg-E mice, but non-significant in C57BL/6j and DBA/2j.

10-day Agg-E session locomotion: The locomotion of Agg-E mice of C57BL/6j strain showed significant diminution 1 d after the 10-day Agg-E session; the 4 w delay caused a modest recovery. The Agg-E mice of the DBA/2j strain displayed decreased locomotion both 1 d and 4 w after the 10-day Agg-E session, compared to the controls. Moreover, the Agg-E mice of the DBA/2j strain displayed further diminution of locomotion after the 4 w delay. The Agg-E mice of the BALB/cj strain consistently showed more decreased locomotion compared to controls both 1 d and 4 w after the 10-day Agg-E session.

Delay post stress was the significant factor for interpreting the variability in decreased locomotion.

MANOVA analysis suggested significant interactive influence of all three dependent variables.

Considering the three strains individually, the interplay between Stress and Delay emerged as non-significant across the strains.

3.3.6. Overall activity (Fig. S2B, Table 3)

5-day Agg-E session Overall activity: The *overall activities* of C57Bl/6j Agg-E mice were similar to the controls 1 d after the 5-day Agg-E session, and decreased marginally after 10 d delay. In DBA/2j, the activity level was significantly lower than the controls 1 d after the 5-day Agg-E session, and subsequent 1.5 w delay resulted in the activity level returned to the baseline. As in the case of C57BL/6j, Agg-E mice of BALB/cj displayed control-like activities 1 d after the 5-day Agg-E session, but BALB/cj showed a significant decrease in activity than controls after 1.5 w delay.

Stress history particularly defined by Stress and its interplay with Strain displayed a modest effect on *overall activity*.

MANOVA analysis suggested moderate interactive influence of all three dependent variables.

Considering the three strains individually, the interplay between Stress and Delay emerged as modestly significant in C57BL/6j [20] and non-significant in the other two strains.

10-day Agg-E session Overall activity: The Agg-E mice of both C57BL/6j and DBA/2j displayed control-like *overall activities* 1 d as well as 4 w after the 10-day Agg-E sessions. The Agg-E mice of BALB/cj strain displayed variable but not significantly elevated activities compared to controls 1 d after the 10-day Agg-E schedule but the increased activity of BALB/cj mice compared to controls reached statistical significance after 4 w delay.

None of the factors and their interplays was significant in interpreting overall activity as inferred by 2-/3-way ANOVA and MANOVA.

Considering the three strains individually, the interplay between Stress and Delay emerged as non-significant across the strains.

3.4. Cardio-histopathological analysis of the Agg-E mice

We examined the heart tissue for two potential indicators of cardiac dysfunctions typically induced by stress, myocardial degeneration and lymphohistiocytic myocarditis ($N = 5$ per group) [21]. More than 12% of the C57BL/6j Agg-E mice displayed myocardial degeneration 1 d after the 5-day Agg-E session. This myopathy became more pronounced after 1.5 w delay as 60% of the Agg-E mice sustained myocardial degeneration. 10-day Agg-E session caused myocardial degeneration among 36.4% of C57BL/6j Agg-E mice. Four week delay may have permitted some reversal of the atrophy, as only 20% of the Agg-E mice displayed myocardial degeneration. We diagnosed lymphohistiocytic myocarditis in 75% and 45.5% of C57BL/6j Agg-E mice 1 d after the 5-day and 10-day Agg-E sessions, respectively. The subsequent delays might have positive impact on this inflammation.

The cardiac health of DBA/2j mice was found least susceptible to this type of stress as none of the DBA/2j Agg-E mice displayed any cardiac dysfunction after the Agg-E schedule.

One day after the 5-day Agg-E session, 36.4% of BALB/cj Agg-E mice displayed myocardial degeneration, but none of them showed lymphohistiocytic myocarditis. No cardiac myopathy was diagnosed after the delay periods.

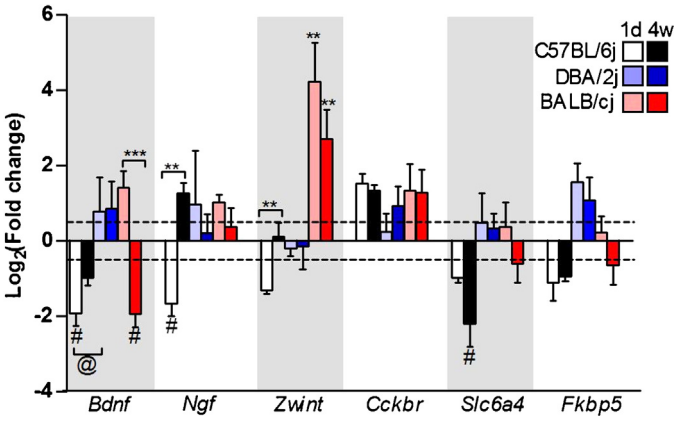

Fig. 3. qPCR result of the selected panel of genes. The fold changes (Agg-E mice/Control) of six genes are reported in $\log_2$ scale. The horizontal dotted line passing through 1.0 of *Y*-axis marks the cut-off at fold change ±1.5. Welch's correction compared Agg-E mice vs. control mice: $^{\#}p < 0.05$; 1 d vs. 4 w: @ $0.05 < p < 0.1$, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.0001$.

3.5. Real-time PCR results to characterize the selective genetic responses to repeated Agg-E stress

We measured the regulations of six genes using qPCR, namely *Bdnf*, *Ngf*, *Zwint*, *Cckbr*, *Slc6a4* and *Fkbp5* (Fig. 3). Table 4 documents a brief literature survey linking these molecules to PTSD and synaptic plasticity. For this study, we used hemibrains collected from Agg-E ($N = 4–8$) and control mice ($N = 4–10$) of all three strains subjected to 10-day Agg-E sessions and euthanized either 1 d or 4 w post-stress.

Bdnf: One day after a 10-day Agg-E session, *Bdnf* was about four-fold suppressed in C57BL/6j Agg-E mice. Four weeks delay shifted this to nearly two-fold down-regulated from the control level. In DBA/2j, *Bdnf* was elevated by nearly two-fold among Agg-E mice 1 d after the 10-day Agg-E session and remained at this level 4 w post-stress. We observed a 2.5-fold elevation of *Bdnf* in BALB/cj Agg-E mice 1 d after the 10-day stress that reduced significantly 4 w post-stress.

Ngf: In C57BL/6j Agg-E mice, the down-regulated expression of *Ngf* 1 d after the 10-day Agg-E session became up regulated after 4 w delay. Two-fold elevated expressions were observed in Agg-E mice of the other two strains 1 d post-Agg-E session that declined to the control level after 4 w delay.

Zwint: In C57BL/6j Agg-E mice, *Zwint* was significantly suppressed 1 d after the 10-day Agg-E session and was elevated to control level 4 w post-stress. A control-like expression was observed in DBA/2j Agg-E mice. A consistently and significantly elevated expression of *Zwint* was observed among BALB/cj Agg-E mice.

Cckbr: One day after the 10-day Agg-E session, *Cckbr* was almost significantly elevated in C57BL/6j Agg-E mice, and after 4 w it remained 2.5-fold elevated from the control. A late and nearly two-fold elevation was observed in DBA/2j Agg-E 4 w after the 10-day Agg-E session. There was a consistent two-fold elevation in BALB/cj Agg-E mice 1 d and 4 w after the 10-day Agg-E session.

Slc6a4: Consistent suppression of *Slc6a4 w*as observed in C57BL/6j Agg-E mice, and even more so after 4 w delay. In contrast, a control-like expression was observed in DBA/2j Agg-E mice 1 d and 4 w after the 10-day Agg-E session. Among BALB/cj Agg-E mice, we found control-like regulation 1 d after the 10-day Agg-E session followed by slight suppression 4 w later.

Fkbp5: A consistent two-fold suppression of *Fkbp5* was registered 1 d and 4 w post-10-day Agg-E session in C57BL/6j Agg-E mice. In DBA/2j Agg-E mice, *Fkbp5* remained two-fold elevated 1 d and 4 w post-10-day Agg-E session. A slight suppression was observed in BALB/cj Agg-E mice 4 w post-10-day Agg-E session.

Table 4
A brief literature survey linking the panel of genes of interest to PTSD and co-morbidities.

Brain derived neurotrophic factor (*BDNF*)	*Human:* Recent PTSD patients have higher *BDNF* than long term PTSD patients likely due to the biased consolidation of fear memory [55]. *Rat:* Contextual fear causes exon-specific *BDNF* expression [66]. *Rat:* Sleep deprivation, western diet and predator scent stress suppress hippocampal *BDNF* [62,63].
Nerve growth factor (NGF)	*Mouse:* NGF depletion contributes to age related cognitive dysfunction [67]. *Mouse: NGF* in hippocampus and amygdala is negatively related to anxiety but positively related to the conditioned fear [68].
ZW10 interactor (ZWINT)	*Mammalian cells*: *ZWINT* is involved in pre-synaptic events in vitro [69]. *Rat:* ZWINT helps in neuroprotection against sub-lethal stress in vitro [70].
Cholecystokinin B receptor (CCKBR)	*Human: CCKBR* is associated with panic disorder [59]. *Mouse:* CCKBR is responsible for anxiety-related behavior [76,77].
Solute carrier family 6 (neurotransmitter transporter, serotonin), member 4 (SLC6A4)	*Human:* Suppressed polymorphism in the serotonin transporter gene is associated with PTSD [58]. *Mouse:* Suppressed serotonin transporter gene elevates social avoidance and depression [78].
FK506 binding protein 5 (FKBP5)	*Human: Interaction of FKBP5* with acute child abuse is a putative predictor of adult PTSD [56]. *Human:* Four single nucleotide polymorphisms located within the *FKBP5* are associated with PTSD risk [57]. *Mouse:* FKBP5 is associated with chronic stress [72].

4. Discussion

Previous studies exploited genetic variances among the various mouse strains to understand its contributions in shaping the social behaviors [44], accomplishing the behavioral tasks [45] and responding to various types of stress, such as brief footshock [14,28], predator's scent [29] and early life trauma [46]. The dual effects of the genetic heterogeneity and the phenotypic challenges (such as changing stressor from neurological injury to emotional assault, introducing environmental novelty) on the rodents' response profile (such as fear plasticity, neurotransmission) were clearly evident. In this context, the present objective is to characterize the interplay between the genetic heterogeneity and environmental variables in developing PTSD-like features in murine model.

We used a conspecific Agg-E stress model, a modified version of the aggressor–intruder social stress model [20] potentially simulating the unique experiences of the soldiers, who repeatedly encounter the life threatening trauma in combat theater. Carefully designing the conspecific and repeated interaction with physically threatening traumas, this model may replicate some aspects of combat stress potentially more effectively than the conventional PTSD models such as the brief footshock model (not a prolonged and repeated stress model) and predator scent model (not a conspecific model). The concern [12,26] that repeated social stress may elicit habituation rather than triggering the PTSD-like features was mitigated by carefully implementing the 'randomness' in the occurrence of the life-threatening events. Thereby the 'ethological validity' of the model namely the unpredictable and uncontrollable nature of the PTSD simulating trauma was established. Moreover, the 'dose-response'-type feature was presented as 10-day long

stress in comparison to 5-day long stress emerged more proficient in eliciting PTSD-like features [20–23]. In addition, the 'face validity' of PTSD-like features [12,26] was manifested by introducing the stressed mice to the contextual cues immediately after withdrawing the trauma and at a delayed interval, wherein 4 weeks is arguably equivalent to nearly 3 years of human life. Supporting our claim we demonstrated many traumatic features emerging after a delay following the withdrawal of stress [20–23]. Concluding, the present data display many PTSD-simulating phenotypic and molecular signatures immediately after the trauma-withdrawal; and many of these traits were sustained through, or even emerged after the interval of delay. As a 'construct validity' of the PTSD rodent model many of the reported putative genomic [21], transcriptomic [22], metabolomic [23] and cellular [20] signatures of PTSD were found perturbed in the present Agg-E repeated stress model. Accordingly, this rodent model meaningfully simulates the combat related PTSD paradigm and validated some of the essential criteria of PTSD rodent models.

Employing this model, we explored genetic heterogeneity of three in-bred strains of mice for stress-elicited display of PTSD-like phenotypes. Sub-population analysis based on the individual variability in responding to Agg-E stress is however beyond the scope of present study. The sample size used herein was optimum for the phenotypic assays, however the size of the subgroups were often below to draw any biologically meaningful inferences; therefore carrying out such computation was overruled for the present purpose.

4.1. DBA/2j displayed a different strategy to cope with stress

Compared to C57BL/6j, DBA/2j displayed an alteration of phenotypes, such as *jumping*, *fightback* and *freezing* with of 10-day Agg-E stress. Consistently robust jumping helped DBA/2j to evade Agg attack, which was accompanied with a gradual decrease of *fightback*, to display a very different strategy to evade Agg mice.

B*odyweight w*as a superior physiological signature of Agg-E stress compared to *temperature*. The pre-stress *bodyweight* is the preferred signature probably for the following reasons. The plasticity of the post-stress bodyweight could be likely due to the immediate effect of 6 h starvation than potential metabolic irregularities caused by any adjustment of the rodent's dietary practice. C57BL/6j and BALB/cj mice displayed opposite trends in both pre-and post-stress *bodyweights*, supporting a past report [25]. Significant strain-to-strain variability in bodyweight accounted for the impact of genetic predisposition in controlling metabolism under stress.

Decreasing territorial behavior correlated with waning *fightback* could be due to the nature of BALB/cj Agg-E mice conceding to the aggressor. In contrast, DBA/2j's display of increasing territoriality might have signified its improved assessment of the threat; and simultaneous reduction of *fightback* suggested that it potentially adopted a different strategy to evade the Agg mice compared to the other strains.

4.2. C57BL/6j displayed pronounced cardiomyopathy

A previous study investigating inter-strain cardiac health found C57BL/6j in particular was an outlier in demonstrating significant susceptibility to hypoxic challenges [47]. The causal relationship between hypoxia and depression, a major PTSD-comorbidity, should be noted in this context [48]. In accordance with these findings, we observed profound adverse impacts of the Agg-E stress on the C57BL/6j Agg-E mice, and its genomic consequences have been investigated by us in a previous study, limited to just one strain [21]. Five-day Agg-E sessions caused a late augmentation of myocardial degeneration, while 10-day Agg-E sessions caused a rather consistent cardiomyopathy. The increased bodyweight of C57BL/6j could have a causal relationship with its poor cardiac health, as previous studies suggested close associations among obesity/metabolic disorder, cardiac health and PTSD symptoms [49,50]. In the present study we observed 75% of C57BL/6j mice developed lymphohistiocytic myocarditis, and none of DBA/2j did so, and while 36.4% of BALB/cj showed an immediate effect after 10 d Agg-E stress.

4.3. Three strains displayed different patterns of coping with Agg-E stress over time

Fixation to a past traumatic event and reemergence of anxiety, depression and other stress-associated phenotypes elicited by contextual provocation are some of the major traits of PTSD [12], which were simulated in the present study by monitoring six behavioral endpoints (Table 1) during the partition test [20]. The *partition vigilance* and *withdrawal time in the periphery* counterbalances the curiosity against the tendency of dissociation from potential threat. The variances in both traits after 5-day and 10-day Agg-E sessions were explained by the significant interaction between the strain differences and the Stress history. A delayed increase in *partition avoidance* observed in BALB/cj potentially captured the time-evolved emergence of the rodent's fear response to the traumatic cue. Since the delayed emergence (or re-experiencing) of trauma is an essential criterion of PTSD [51,52], the delayed onset of PTSD-like features are of particular interest.

Grooming, an accepted signature of anxiety, depression and hyperarousal [53] and was altered by the interaction between Strain and Stress history after the 10-day Agg-E session. Re-experiencing the contextual fear, BALB/cj Agg-E mice spent significantly more time grooming 4 w after the 10-day Agg-E session.

Freezing and *reduced locomotion* are the dual acts of fear response, possibly caused by an overcautious approach to potential danger and the tendency of conceding to the traumatic context [54]. The significant interplay between strain difference and Stress history explained the variance in freezing after the 10-day Agg-E session. Once again, BALB/cj Agg-E mice spent significantly more time freezing 4 w after the 10-day Agg-E session.

It is important to note that the *overall activities* of Agg-E mice were almost control-like except for BALB/cj mice whose possible 'anxious' behavior manifested by frequent *grooming* might have contributed to their apparent hyperactivity. The control-like activities displayed by the other two strains in general suggested that their hypoactivities, such as *freezing* and *retarded locomotion*, were potentially driven by the contextual fear, not by lethargy or habituation.

Mapping of these behavioral parameters in PCA (Fig. S4 and Tables S2–3) revealed similar longitudinal trajectories of coping with the stress manifested by C57BL/6j and DBA/2j post-5-day Agg-E session, whereas, BALB/cj displayed a reversed longitudinal trajectory. DBA/2j Agg-E mice maintained a minimal behavioral shift post-10-day Agg-E session, depicting a delay-independent response to Agg-E stress.

The behavioral changes between day 1 and day 10 were strikingly different between C57BL/6j and BALB/cj for both vigilance and freezing – almost mirror images of one another. Vigilance was increasing in C57BL/6j but decreasing in BALB/cj. In marked contrast, freezing was decreasing in C57BL/6j as it was increasing in BALB/cj. The reemergence or delayed development of many psychiatric symptoms is typical among PTSD patients [51,52], therefore these distinctive longitudinal patterns of behavioral shifts demonstrated by BALB/cj would be important to examine further.

4.4. C57Bl/6j and BALB/cj displayed perturbed expression of selected PTSD signatures

Genes associated with synaptic plasticity (*Bdnf*, *Ngf* and *Zwint*) and genes encoding receptors for cholcystokinin (e.g. *Cckbr*), co-chaperone of the glucocorticoid (e.g. *Fkbp5*) and transporter of serotonin (e.g. *Slc6a4*) were evaluated. Potential association of *Bdnf* [55], *Fkbp5* [56,57] and *Slc6a4* [11,58] with PTSD has been established, while *Cckbr* has been linked to anxiety [59]. The measurements in the present study were limited for being focused on the hemibrains, not on the different brain regions. Furthermore, the outcome could be potentially compromised by the collection of the small midline structures such as thalamic and hypothalamic nuclei.

The complex relationship of *Bdnf* with synaptic plasticity, depression and memory consolidation [60,61] might be taken into account to explain its variable expression across the strains. We observed suppressed *Bdnf* in C57BL/6j in accordance with the studies probing sleep deprivation [62], predator stress [63], acute social defeat [64] and social isolation [65]. Exon-specific elevation of *Bdnf* in rat hippocampus, however, was reported as a consequence of restraint stress [66]. In comparison to patients with long-standing PTSD, patients with recently acquired PTSD have been diagnosed with higher serum *Bdnf*, which was interpreted as a causal consequence of the biased consolidation of fear memory [55]. This observation may explain the elevated *Bdnf* found immediately after 10-day Agg-E stress in BALB/cj, followed by delayed suppression. Hence, frequent manifestation of delayed arousal of PTSD-like traits in BALB/cj such as *withdrawal to periphery*, *freezing*, and *grooming* could be attributed to the biased consolidation of fear memory causally related to the suppression of *Bdnf* in hemibrain.

Significant changes of *Ngf* were limited to C57BL/6j, where there was a decrease on day 1 followed by an increase on day 10. Initial suppression of *Ngf* in C57BL/6j might hypothetically be associated with cognitive dysregulation [67], increased anxiety and poor fear conditioning [68] as the immediate effect of 10-day Agg-E stress. Subsequent elevation of *Ngf* after 4 w delay, associated with decreased withdrawal and decreased freezing putatively suggests the molecule's association with reversal of fear conditioning [68]. *Zwint*, another gene closely associated with synaptic plasticity [69,70], was suppressed but only on day one in C57BL/6j. *Zwint* showed a significant elevation at both time points in BALB/cj, indicating a possible strain-specific and stress-induced prolonged alteration of synaptic plasticity. In accordance with their endophenotypes, DBA/2j displayed minimum perturbation of *Bdnf*, *Ngf* and *Zwint*, which might be associated with its apparent resilience in the paradigm. Onset of stress typically diminishes the activity of the co-chaperone domain FKBP5 [71], which has been shown in the chronic stress model [72] and presented in the Agg-E model assessing C57BL/6j and BALB/cj.

4.5. Summary

The patterns of response to Agg-E stress emerged significantly distinct among the strains. C57Bl/6j showed very poor cardiac health and a very different phenotypic strategy of coping with Agg-E stress, compared to other strains. Delayed emergence of many PTSD-simulating features distinguished BALB/cj from the other two strains, with exception of myocardial vulnerability, which was expressed early. The strain-to-strain variability in characterizing the PTSD-like phenotypes was primarily explained by the interplay of the genetic heterogeneity interacting with stress history. The contribution of Stress history was primarily defined by the magnitude of the stress and its interplay with the delay period (Stress × Delay).

It is further interesting to note that there is a close association of the regulation of a selected pool of genes with phenotypic plasticity. Specifically, the comparatively distant molecular expressions seen in DBA/2j were in contrast with the other two strains. Studies identifying phenotype-specific molecular footprints [66,73,74] are underway; more comprehensive approaches, with a focus on investigating individual brain regions in respect to PTSD models are warranted.

Disclaimer

The views, opinions, and/or findings contained in this report are those of the authors and should not be construed as official Department of the Army position, policy, or decision, unless so designated by other official documentation.

Research was conducted in compliance with the Animal Welfare Act, and other Federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the Guide for the Care and Use of Laboratory Animals (NRC, 2011) in facilities that are fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care, International.

Citations of commercial organizations or trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbr.2015.05.038

References

[1] Jaffee SR, Price TS. Gene–environment correlations: a review of the evidence and implications for prevention of mental illness. Mol Psychiatry 2007;12(5):432–42.
[2] Kendler KS, Eaves LJ. Models for the joint effect of genotype and environment on liability to psychiatric illness. Am J Psychiatry 1986;143(3):279–89.
[3] Stein MB, Jang KL, Taylor S, Vernon PA, Livesley WJ. Genetic and environmental influences on trauma exposure and posttraumatic stress disorder symptoms: a twin study. Am J Psychiatry 2002;159(10):1675–81.
[4] Goldberg J, True WR, Eisen SA, Henderson WG. A twin study of the effects of the Vietnam War on posttraumatic stress disorder. JAMA 1990;263(9):1227–32.
[5] Sartor CE, et al. Common genetic and environmental contributions to posttraumatic stress disorder and alcohol dependence in young women. Psychol Med 2011;41(7):1497–505.
[6] Yehuda R, et al. Parental posttraumatic stress disorder as a vulnerability factor for low cortisol trait in offspring of holocaust survivors. Arch Gen Psychiatry 2007;64(9):1040–8.
[7] Berkowitz SJ, Stover CS, Marans SR. The Child and Family Traumatic Stress Intervention: secondary prevention for youth at risk of developing PTSD. J Child Psychol Psychiatry Allied Discip 2011;52(6):676–85.
[8] Gauderman WJ. Sample size requirements for matched case-control studies of gene–environment interaction. Stat Med 2002;21(1):35–50.
[9] Hetherington MM, Cecil JE. Gene–environment interactions in obesity. Forum Nutr 2010;63:195–203.
[10] Lau JY, Eley TC. Gene–environment interactions and correlations in psychiatric disorders. Curr Psychiatry Rep 2004;6(2):119–24.
[11] Kolassa IT, et al. Association study of trauma load and SLC6A4 promoter polymorphism in posttraumatic stress disorder: evidence from survivors of the Rwandan genocide. J Clin Psychiatry 2010;71(5):543–7.
[12] Siegmund A, Wotjak CT. Toward an animal model of posttraumatic stress disorder. Ann NY Acad Sci 2006;1071:324–34.
[13] Wimer RE, Wimer CC, Roderick TH. Genetic variability in forebrain structures between inbred strains of mice. Brain Res 1969;16(1):257–64.
[14] Ammassari-Teule M, Passino E, Restivo L, de Marsanich B. Fear conditioning in C57/BL/6 and DBA/2 mice: variability in nucleus accumbens function according to the strain predisposition to show contextual- or cue-based responding. Eur J Neurosci 2000;12(12):4467–74.
[15] Airey DC, Robbins AI, Enzinger KM, Wu F, Collins CE. Variation in the cortical area map of C57BL/6J and DBA/2J inbred mice predicts strain identity. BMC Neurosci 2005;6:18.
[16] Mandel P, Ayad G, Hermetet JC, Ebel A. Correlation between choline acetyltransferase activity and learning ability in different mice strains and their offspring. Brain Res 1974;72(1):65–70.

[17] Good RL, Liang LP, Patel M, Radcliffe RA. Mouse strain- and age-dependent effects of binge methamphetamine on dopaminergic signaling. Neurotoxicology 2011;32(6):751–9.
[18] Fairless AH, et al. Sociability and brain development in BALB/cJ and C57BL/6J mice. Behav Brain Res 2012;228(2):299–310.
[19] Papaleo F, Lipska BK, Weinberger DR. Mouse models of genetic effects on cognition: relevance to schizophrenia. Neuropharmacology 2012;62(3):1204–20.
[20] Hammamieh R, et al. Murine model of repeated exposures to conspecific trained aggressors simulates features of post-traumatic stress disorder. Behav Brain Res 2012;235(1):55–66.
[21] Cho JH, et al. Molecular evidence of stress-induced acute heart injury in a mouse model simulating posttraumatic stress disorder. Proc Natl Acad Sci U S A 2014.
[22] Seid Muhie AG, James Meyerhoff, Nabarun Chakraborty, Rasha Hammamieh, Marti Jett. Brain transcriptome profiles in mouse model simulating features of post-traumatic stress disorder. Mol Brain 2015;8.1:14. PMID: 25888136.
[23] Gautam A, et al. Acute and chronic plasma metabolomic and liver transcriptomic stress effects in a mouse model with features of post-traumatic stress disorder. PLOS ONE 2015;10(1):e0117092.
[24] Krishnan V, et al. Molecular adaptations underlying susceptibility and resistance to social defeat in brain reward regions. Cell 2007;131(2):391–404.
[25] Razzoli M, Carboni L, Andreoli M, Ballottari A, Arban R. Different susceptibility to social defeat stress of BalbC and C57BL6/J mice. Behav Brain Res 2011;216(1):100–8.
[26] Siegmund A, Wotjak CT. A mouse model of posttraumatic stress disorder that distinguishes between conditioned and sensitised fear. J Psychiatr Res 2007;41(10):848–60.
[27] Matar MA, Zohar J, Cohen H. Translationally relevant modeling of PTSD in rodents. Cell Tissue Res 2013;354(1):127–39.
[28] Szklarczyk K, Korostynski M, Golda S, Solecki W, Przewlocki R. Genotype-dependent consequences of traumatic stress in four inbred mouse strains. Genes Brain Behav 2012.
[29] Belzung C, El Hage W, Moindrot N, Griebel G. Behavioral and neurochemical changes following predatory stress in mice. Neuropharmacology 2001;41(3):400–8.
[30] Tang X, Orchard SM, Sanford LD. Home cage activity and behavioral performance in inbred and hybrid mice. Behav Brain Res 2002;136(2):555–69.
[31] Podhorna J, Brown RE. Strain differences in activity and emotionality do not account for differences in learning and memory performance between C57BL/6 and DBA/2 mice. Genes Brain Behav 2002;1(2):96–110.
[32] Hammamieh R, et al. Transcriptomic analysis of the effects of a fish oil enriched diet on murine brains. PLOS ONE 2014;9(3):e90425.
[33] Lumley LA, Sipos ML, Charles RC, Charles RF, Meyerhoff JL. Social stress effects on territorial marking and ultrasonic vocalizations in mice. Physiol Behav 1999;67(5):769–75.
[34] Carola V, D'Olimpio F, Brunamonti E, Mangia F, Renzi P. Evaluation of the elevated plus-maze and open-field tests for the assessment of anxiety-related behaviour in inbred mice. Behav Brain Res 2002;134(1–2):49–57.
[35] Clement Y, et al. Anxiety in mice: a principal component analysis study. Neural Plas 2007;2007:35457.
[36] Moura PJ, Meirelles ST, Xavier GF. Long-term social recognition memory in adult male rats: factor analysis of the social and non-social behaviors. Braz J Med Biol Res = Revista brasileira de pesquisas medicas e biologicas/Sociedade Brasileira de Biofisica. . . [et al] 2010;43(7):663–76.
[37] O'Leary TP, Gunn RK, Brown RE. What are we measuring when we test strain differences in anxiety in mice? Behav Genet 2013;43(1):34–50.
[38] Kanari K, Kikusui T, Takeuchi Y, Mori Y. Multidimensional structure of anxiety-related behavior in early-weaned rats. Behav Brain Res 2005;156(1):45–52.
[39] Tanaka S, Young JW, Halberstadt AL, Masten VL, Geyer MA. Four factors underlying mouse behavior in an open field. Behav Brain Res 2012;233(1):55–61.
[40] Lassance-Soares RM, et al. Chronic stress impairs collateral blood flow recovery in aged mice. J Cardiovasc Transl Res 2014;7(8):749–55.
[41] Logan RW, Nicole Edgar, Gillman AG, Daniel Hoffman, Xiyu Zhu, McClung CA. Chronic stress induces brain region specific alterations of molecular rhythms in mice that correlate with depression-like behavior. Biol Psychiatry 2015. PMID: 25771506, (Epub ahead of print).
[42] Koolhaas JM, et al. Stress revisited: a critical evaluation of the stress concept. Neurosci Biobehav Rev 2011;35(5):1291–301.
[43] Philbert J, et al. Acute inescapable stress exposure induces long-term sleep disturbances and avoidance behavior: a mouse model of post-traumatic stress disorder (PTSD). Behav Brain Res 2011;221(1):149–54.
[44] Sankoorikal GM, Kaercher KA, Boon CJ, Lee JK, Brodkin ES. A mouse model system for genetic analysis of sociability: C57BL/6J versus BALB/cJ inbred mouse strains. Biol Psychiatry 2006;59(5):415–23.
[45] Tucci V, et al. Gene–environment interactions differentially affect mouse strain behavioral parameters. Mamm Genome 2006;17(11):1113–20.
[46] Holmes A, et al. Early life genetic, epigenetic and environmental factors shaping emotionality in rodents. Neurosci Biobehav Rev 2005;29(8):1335–46.
[47] Barnabei MS, Palpant NJ, Metzger JM. Influence of genetic background on ex vivo and in vivo cardiac function in several commonly used inbred mouse strains. Physiol Genomics 2010;42A(2):103–13.
[48] Takano T, et al. Cortical spreading depression causes and coincides with tissue hypoxia. Nat Neurosci 2007;10(6):754–62.
[49] Rasmusson AM, Schnurr PP, Zukowska Z, Scioli E, Forman DE. Adaptation to extreme stress: post-traumatic stress disorder, neuropeptide Y and metabolic syndrome. Exp Biol Med 2010;235(10):1150–62.
[50] Kubzansky LD, Koenen KC. Is post-traumatic stress disorder related to development of heart disease? Future Cardiol 2007;3(2):153–6.
[51] Association AP. Diagnostic Criteria from DSM-IV. Washington, DC: The Association; 1994.
[52] Pamplona FA, et al. Prolonged fear incubation leads to generalized avoidance behavior in mice. J Psychiatr Res 2011;45(3):354–60.
[53] Kalueff AV, Tuohimaa P. Experimental modeling of anxiety and depression. Acta Neurobiol Exp (Wars) 2004;64(4):439–48.
[54] Golub Y, et al. Reduced hippocampus volume in the mouse model of posttraumatic stress disorder. J Psychiatr Res 2011;45(5):650–9.
[55] Hauck S, et al. Serum brain-derived neurotrophic factor in patients with trauma psychopathology. Prog Neuro-psychopharmacol Biol Psychiatry 2010;34(3):459–62.
[56] Binder EB, et al. Association of FKBP5 polymorphisms and childhood abuse with risk of posttraumatic stress disorder symptoms in adults. JAMA 2008;299(11):1291–305.
[57] Boscarino JA, Erlich PM, Hoffman SN, Zhang X. Higher FKBP5, COMT, CHRNA5, and CRHR1 allele burdens are associated with PTSD and interact with trauma exposure: implications for neuropsychiatric research and treatment. Neuropsychiatr Dis Treat 2012;8:131–9.
[58] Kilpatrick DG, et al. The serotonin transporter genotype and social support and moderation of posttraumatic stress disorder and depression in hurricane-exposed adults. Am J Psychiatry 2007;164(11):1693–9.
[59] Muinos-Gimeno M, et al. Human microRNAs miR-22, miR-138-2, miR-148a, and miR-488 are associated with panic disorder and regulate several anxiety candidate genes and related pathways. Biol Psychiatry 2011;69(6):526–33.
[60] Zagrebelsky M, et al. The p75 neurotrophin receptor negatively modulates dendrite complexity and spine density in hippocampal neurons. J Neurosci 2005;25(43):9989–99.
[61] Woo NH, et al. Activation of p75NTR by proBDNF facilitates hippocampal long-term depression. Nat Neurosci 2005;8(8):1069–77.
[62] Alzoubi KH, Khabour OF, Salah HA, Abu Rashid BE. The combined effect of sleep deprivation and Western diet on spatial learning and memory: role of BDNF and oxidative stress. J Mol Neurosci 2013;50(1):124–33.
[63] Kozlovsky N, et al. Long-term down-regulation of BDNF mRNA in rat hippocampal CA1 subregion correlates with PTSD-like behavioural stress response. Int J Neuropsychopharmacol 2007;10(6):741–58.
[64] Pizarro JM, et al. Acute social defeat reduces neurotrophin expression in brain cortical and subcortical areas in mice. Brain Res 2004;1025(1–2):10–20.
[65] Berry A, et al. Social deprivation stress is a triggering factor for the emergence of anxiety- and depression-like behaviours and leads to reduced brain BDNF levels in C57BL/6J mice. Psychoneuroendocrinology 2012;37(6):762–72.
[66] Takei S, et al. Enhanced hippocampal BDNF/TrkB signaling in response to fear conditioning in an animal model of posttraumatic stress disorder. J Psychiatr Res 2011;45(4):460–8.
[67] Terry Jr AV, Kutiyanawalla A, Pillai A. Age-dependent alterations in nerve growth factor (NGF)-related proteins, sortilin, and learning and memory in rats. Physiol Behav 2011;102(2):149–57.
[68] Yee BK, Zhu SW, Mohammed AH, Feldon J. Levels of neurotrophic factors in the hippocampus and amygdala correlate with anxiety- and fear-related behaviour in C57BL6 mice. J Neural Transm 2007;114(4):431–44.
[69] Endo H, Ikeda K, Urano T, Horie-Inoue K, Inoue S. Terf/TRIM17 stimulates degradation of kinetochore protein ZWINT and regulates cell proliferation. J Biochem 2012;151(2):139–44.
[70] Dai C, et al. Functional identification of neuroprotective molecules. PLoS ONE 2010;5(11):e15008.
[71] Binder EB. The role of FKBP5, a co-chaperone of the glucocorticoid receptor in the pathogenesis and therapy of affective and anxiety disorders. Psychoneuroendocrinology 2009;34(Suppl. 1):S186–95.
[72] Hartmann J, et al. The involvement of FK506-binding protein 51 (FKBP5) in the behavioral and neuroendocrine effects of chronic social defeat stress. Neuropharmacology 2012;62(1):332–9.
[73] Gale GD, et al. A genome-wide panel of congenic mice reveals widespread epistasis of behavior quantitative trait loci. Mol Psychiatry 2009;14(6):631–45.
[74] Han HJ, et al. Strain background influences neurotoxicity and behavioral abnormalities in mice expressing the tetracycline transactivator. J Neurosci 2012;32(31):10574–86.
[75] Vergnes M, Depaulis A, Boehrer A. Parachlorophenylalanine-induced serotonin depletion increases offensive but not defensive aggression in male rats. Physiol Behav 1986;36(4):653–8.
[76] Miyasaka K, et al. Anxiety-related behaviors in cholecystokinin-A, B, and AB receptor gene knockout mice in the plus-maze. Neurosci Lett 2002;335(2):115–8.
[77] Chen Q, Nakajima A, Meacham C, Tang YP. Elevated cholecystokininergic tone constitutes an important molecular/neuronal mechanism for the expression of anxiety in the mouse. Proc Natl Acad Sci U S A 2006;103(10):3881–6.
[78] Bartolomucci A, et al. Increased vulnerability to psychosocial stress in heterozygous serotonin transporter knockout mice. Dis Model Mech 2010;3(7–8):459–70.